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Micro Scoop Cantilever: New Manipulation Technique for Cell Biophysics
Takahiro W. Nakayama, Shin-ichi Machida, Masakazu Saito, Rehana Afrin, Atsushi Ikai.
Mechanical properties surrounding cells are well known to affect behaviors of intracellular and extracellular proteins, and contribute to macroscopically observable biological phenomena. On studying cellular response to the applied mechanical environment, quantitative measurement of the relationship between the applied load and cellular response is important. The atomic force microscope (AFM) can help us to obtain required data for such analysis covering a wide range of force required for such study. When used as a colloidal probe, a bead is glued to a conventional AFM cantilever in advance and used for cell binding experiment only once providing a low throughput experiment. We designed a new cantilever with a scooping function which can produce high throughput cell binding experiments because the colloidal probes are separated from the cantilever. The newly designed AFM cantilever has a semi-circular hole at its edge for scooping function of a spherical bead, thus, micro scoop cantilever. The micro scoop cantilever allowed us to perform various experiments such as recovery of proteins interacting with ligands on a bead, detection of bead adhesion force up to a micronewton order, and simultaneous measurements of loading force to cell and local cellular mechanical responses.

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High-Frequency Electromagnetic Dynamics Properties of THP1 Cells Using Scanning Microwave Microscopy
Yoo Jin Oh, Hans-Peter Huber, Markus Hochleitner, Memed Duman, Bianca Bozna, Markus Kastner, Ferry Kienberger, Peter Hinterdorfer.
Microwave measurements combined with scanning probe microscopy is a novel tool to explore high-localized mechanical and electrical properties of biological species. Complex permittivities and permeabilities are detected through slight variations of an incident microwave signal. Here we report the high-frequency dependence of the electromagnetic dynamic characteristics in human monocytic leukemia cells (THP1) through local measurements by scanning microwave microscopy (SMM). The amplitude and phase images were shown to depend on the applied resonance frequency. While the amplitude yields information about the resistivity determined by the water and the ionic strength, the phase information reflects the dielectric losses arising from the fluid density.

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***A Tale of a Tail* - making Sense of the Shape of Rupture Force Distributions in Single Protein Dissociation Measurements**
Peter M. Hoffmann, Essa Mayyas, Margarida Bernardo, Anjum Sohail, Rafael Fridman.
Atomic force microscopy has been used extensively to measure dissociation in protein systems at the single molecule level. The basic theory, based on a thermal activation and the deformation of the barrier through the applied force, results in a probability distribution for the bond rupture force which is skewed to the left, i.e. towards lower applied force. However, the majority of published measurements show a distinct tail to the right, i.e. the force histograms are skewed towards high force. This ''mystery tail'' has been variously ignored, or assigned to either ''hidden'' multiple attachment events or heterogeneity in the bonding. Multiple attachments can be minimized by using double tethers and discarding any force curves that show too short a contour length for the tether (corresponding to non-specific binding), or clearly show multiple rupture events in the same force curve. However, even with these precautions, multiple ruptures can occur at the same location and appear as a single unbinding events. We have developed a methodology to estimate the frequency of such ''hidden'' multiple rupture events. Using this methodology, we have used the avidin-biotin model system, and prepared samples with varying densities of active sites. This way we controlled the probability of multiple bonding events. From these measurements we were able to determine the relative importance of hidden multiple rupture events versus bond heterogeneity in explaining the unexpected high force tail in rupture force histograms.

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Nanometric Ion Sensing using Near-Field Ratiometric Fluorescence Sensing
Aaron Lewis, Ella Wajnryt, Patricia Hamra, Chaya Lewis.
A nanometric measurement of ionic concentrations at distances extending from nanometers to microns from a charged surface immersed in solution is described. AFM & NSOM techniques are combined using NSOM's nanometric light confinement abilities for optical pH sensing. AFM allowed for knowledge/control of the distance between the optical pH-meter and investigated surface. A ratiometric method of fluorescence sensing was used. A pH sensing fluorescent dye, ie., fluorescein was complexed to a non-pH sensing dye, rhodamine through a dextran molecule and thus the concentration of dye molecules was equivalent in each nanometric solution volume element. The results profile the nanometric variation in pH as a function of distance from such a charged surface. The approach has great potential to generally and accurately monitor nanometrically charge distribution in solutions and in close proximity to surfaces. The measurements provide important experimental underpinnings to long established solution structure theories, eg. those of Debye-Huckel and Gouy-Champman.

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Controlled Immobilization of Proteins at the Nanoscale for Highly Sensitive Immuno-Assay
Loredana Casalis, Fouzia Bano, Barbara Sanavio, Ljiljana Fruk, Stefania Corvaglia, Christof M. Niemeyer, Giacinto Scoles.
The simultaneous identification and quantification of several different proteins is crucial for the understanding of cellular processes at the protein level, and for detecting changes in protein expression signatures due to disease, drugs or any biological perturbation. High-throughput, high sensitivity, nanotechnology-based analytical tools, with low requirements on the sample amount and hands-on processing time, are prerequisites for further developments in this field.
We use atomic force microscopy nanografting (AFM-NG) to fabricate innovative nano-arrays for immuno-assay, with complete and efficient control on protein/antibody immobilization. By combining NG and DNA-directed immobilization (DDI), different protein-ssDNA conjugates are driven simultaneously on top of a nano-array of complementary ssDNA strands, previously prepared on a gold film surface functionalized with a protein repellent reference carpet. The integrity and biochemical functionality of the immobilized proteins were demonstrated as a proof of principle by AFM topography measurements and immunological assays. Different proteins were detected within a complex matrix of standardized human serum, demonstrating the specificity of the biomolecular recognition, and the absence of any nonspecific binding and cross-reactivity. Protein folding was tested by studying protein-ligand interactions.
In a parallel approach we promote protein immobilization in controlled orientation, by means of nitrilotriacetic acid (NTA)-terminated thiols. NTA binds specifically to a polyhistidine tag of the protein (through Ni chelation), which can be genetically engineered in recombinant proteins in a known position. This immobilization is reversible and ensures surface re-usability. Technically, the know-how for both DNA and NTA nanografting based protein immobilization can be combined to exploit the extreme selectivity and parallelizability of the former with the versatility of the latter. Specifically, by using a ssDNA-NTA conjugate, the added to our protein nano-arrays the opportunity to immobilize in an oriented manner through the histidine tag an enormous variety of proteins.

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High Speed AFM Imaging of Glutamate Receptor Activation
Chandra Ramanujan, Nahoko Kasai, Jelena Baranovic, Keiichi Torimitsu, John F. Ryan.
Long-term potentiation (LTP), or enhanced synaptic transmission, is widely believed to be the cellular mechanism that underlies memory formation and learning. It is associated with an increase in activity and number of receptors in the post-synaptic membrane. Fast excitatory neurotransmission is mediated by the glutamate receptor AMPAR (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor), a tetrameric ligand-gated ion channel which consists of four subunits (GluA1-GluA4). In this investigation we have made single molecule measurements of homomeric GluA3 AMPAR reconstituted in mixed-lipid supported membranes (ML-SLM) using both conventional and high-speed atomic force microscopy (AFM) where the image acquisition times are typically 100s and 0.5s respectively. Conformational changes induced by glutamate binding were monitored after UV photolysis of the caged compound.
The ML-SLM exhibit distinct raft formation with the appearance of domains of different thickness. We observe preferential receptor reconstitution into thicker domains with density ~100/μm^2, which is somewhat lower than that found in native postsynaptic membranes, and substantially lower than that in crystals used for protein structure determination. Under our low receptor density conditions the AMPAR extracellular domain observed in AFM is clearly tetrameric, but it appears to be somewhat shorter than expected on the basis of EM and X-ray measurements and more extended laterally in the membrane. Release of glutamate into the imaging buffer results, after a brief time delay, in the appearance of a more spatially open configuration, similar to that observed by negative stain transmission EM. The time delay between glutamate release and the observed change in structure is most likely due to agonist diffusion within the buffer before binding occurs.